Clin Chem Lab Med 2002; 40(6):612–615 © 2002 by Walter de Gruyter · Berlin · New York

The Changes of Superoxide Dismutase, Catalase and Glutathione Peroxidase Activities in Erythrocytes of Active and Passive Smokers[1)]

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Cigarette smoking has been implicated in the pathogenesis of ischemic heart disease, emphysema, obstructive lung disease and neoplastic disorders. More than 1000 constituents of smoke, including many oxidants, pro-oxidants, free radicals and reducing agents, have been identified. The activities of erythrocyte superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px), which are the important components of antioxidant defense system, were measured in 100 healthy volunteers. This study included heavy smokers (consuming cigarettes ≥ 20 per day; n=30, group I), light smokers (consuming cigarettes<20 per day; n=30, group II), passive smokers (exposed to cigarette smoke in the indoor environment; n=20, group III), and non-smokers (n=20, the control group). While activities of SOD and CAT in erythrocytes were significantly lower in groups I, II and III than in the control group ($p<0.01$ for all), mean erythrocyte GSH-Px activity in group III was higher than that in groups I, II and in controls. These results suggest that the increased oxidative stress occurs in smokers, owing to the free radicals present in smoke. It might cause a decrease in antioxidant enzyme activities and oxidant/antioxidant imbalance. We also observed that passive smokers were affected by the environmental smoke to the same extent as active smokers. Clin Chem Lab Med 2002; 40(6):612–615

Key words: Smoking; Superoxide dismutase; Catalase; Glutathione peroxidase.

Abbreviations: CAT, catalase; GSH-Px, glutathione peroxidase; PBS, phosphate buffer saline; SOD, superoxide dismutase.

Introduction

Cigarette smoking has been identified as a significant contributing factor in the etiology of respiratory, cardiovascular and other disorders (1). More than one thousand constituents of smoke, including many oxidants, pro-oxidants, free radicals and reducing agents, have been identified (2, 3). A further oxidative burden can be imposed on smokers as a consequence of a chronic low-level inflammation of the respiratory tract. The number of inflammatory cells in the airways of smokers is increased 2- to 4-fold (4). These cells generate a range of free radicals, mainly oxygen-based, which are linked with their normal role in host defense (1).

Because free radicals are potentially toxic they usually are inactivated or scavenged by antioxidants before they can inflict damage to lipids, proteins or nucleic acids. The human body has a complex antioxidant defense system that includes the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT). These block the initation of free radical chain reactions. The non-enzymatic antioxidant components consist of molecules such as glutathione, α-tocopherol, ascorbic acid and β-carotene that react with activated oxygen species and thereby prevent the propagation of free radical chain reactions. However, when free radicals are generated in excess or the cellular antioxidant defense system is defective, they can stimulate chain reactions by interacting with proteins, lipids and nucleic acids, causing cellular dysfunction and even death (5).

There are contradictory findings on this subject in the literature. For example, increased CAT activity and decreased GSH-Px activity in the erythrocytes have been reported in smokers in one study (6), but in the study of Brown *et al.* (7) erythrocyte antioxidant enzyme activities were found to be increased in smokers. In another study erythrocyte activities of SOD, CAT and GSH-Px were found significantly decreased in smokers (8). In the present study we investigated the effect of smoking and number of cigarettes consumed per day on the activities of the antioxidant enzymes (GSH-Px, CAT and SOD) in erythrocytes, and we also investigated the relationship between these enzymes and the number of cigarettes consumed per day, and smoking duration.

Materials and Methods

This study included 100 healthy subjects (69 male and 31 female, aged 19 to 58 years). All subjects were healthy volunteers recruited from among the hospital employees or their friends. Informed consent was obtained from all subjects. They were not suffering from any diseases and were not on any medication, including vitamins. The subjects were divided into four subgroups: group I (heavy smokers) were individuals smoking ≥ 20 cigarettes per day (n=30), group II (light smokers) were individuals smoking<20 cigarettes per day (n=30), group III (passive smokers) were exposed to passive

[1)] Presented at the 14th IFCC European Congress of Clinical Chemistry and Laboratory Medicine, EUROMEDLAB, in Prague, 26–31 May 2001.

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smoke at work or at home (n=20), and group IV (control group) were non-smokers not exposed to passive smoke at work or at home. The demographic data of the 100 subjects are presented in Table 1.

Venous blood was collected in tubes with acid citrate dextrose and centrifuged at 2500 × *g* for 15 min. Erythrocytes were washed 3 times with equal volumes of phosphate buffer saline (PBS). Then, 0.4 ml of washed erythrocytes was transferred into tubes with 0.4 ml PBS and stored at −80°C until analysis. The remaining blood was collected in plain tubes, without anticoagulant, and centrifuged at 3500 × *g* for 5 min. The serum was used for routine biochemical analysis. Subjects who had abnormal biochemical results were excluded.

Hemoglobin concentration was determined using the cyanmethemoglobin method (9). The principle of SOD activity measurement was based on the inhibition of nitroblue tetrazolium reduction with the xanthine-xanthine oxidase system as a superoxide generator. Superoxide radicals produced reduce nitroblue tetrazolium and form formazane. SOD prevents this reaction, and its activity is inversely proportional to the absorbance value of formazane at 560 nm. By using a blank in which all reagents were present except erythrocytes, and by determining the absorbances of samples and blank, activity was calculated as follows:

Percent inhibition=[$(A_{blank}-A_{sample})/A_{blank}$] × 100.

One SOD unit was defined as the amount of the enzyme causing 50% inhibition of the nitroblue tetrazolium reduction rate. SOD activity was expressed in units per gram hemoglobin (10).

The principle of GSH-Px activity assay was based on the decrease in absorbance of NADPH at 340 nm. GSH-Px oxidizes reduced glutathione to oxidized glutathione which is then reduced by glutathione reductase. In the last reaction, while NADPH is oxidized to $NADP^+$, the absorbance of NADPH at 340 nm begins to decrease steadily. By measuring the absorbance change per minute and by using the molar extinction coefficient of NADPH, GSH-Px activity of erythrocytes is calculated per gram hemoglobin (11).

The principle of the CAT activity assay is based on the determination of the rate constant (s^{-1}, k) of the hydrogen peroxide (H_2O_2) decomposition rate. Rate constant, $k = (1/\Delta t) \times \ln(A_1/A_2)$, where A_1 and A_2, are the absorbance values of H_2O_2 at times t_1 and t_2. CAT activity was expressed as k/g hemoglobin (12). Serum thiocyanate levels were used as an indicator of cigarette smoking.

Results were given as mean ± SD. Erythrocyte SOD, GSH-Px and CAT activities were analyzed by one-way ANOVA and the significance of the mean differences between groups was evaluated by Duncan test. For correlation analysis, Carl Pearson Moment correlation test was used.

Results

The mean activity of SOD, GSH-Px and CAT in the erythrocytes in the different groups are given in Table 2. The results of correlation analysis between the activities of the studied enzymes and between these activities and the number of cigarettes consumed per day and smoking duration are given in Table 3.

As shown in Table 2, there were statistically significant differences between mean activity of the enzymes in most of the groups. In this regard, CAT and SOD activity in the control group and GSH-Px activity in group III were found to be higher than in the other groups. Regarding GSH-Px activities, there were differences between the control group and groups I and II, but these were not significant. While the activity of SOD and CAT in erythrocytes was significantly lower in groups I, II and III than in group IV ($p<0.01$ for all), the mean erythrocyte GSH-Px activity in group III was higher than that of groups I, II and IV ($p<0.01$ for all).

In group I a negative correlation was found between the number of cigarettes consumed per day and erythrocyte CAT activity ($r=-0.34$, $p<0.05$). In group II, a negative correlation was found between erythrocyte SOD activity and the number of cigarettes consumed per day, and smoking duration ($r=-0.36$, $p<0.05$ and $r=-0.40$, $p<0.05$, respectively). There was also a correlation between GSH-Px and the duration of smoking ($r=-0.49$, $p<0.01$). There was a significant correlation between the activity of erythrocyte SOD and GSH-Px in

Table 1 Age and duration of smoking in the study participants.

	Group I (heavy smokers)	Group II (light smokers)	Group III (passive smokers)	Group IV (controls)
Age (years)	32.90±7.25	28.37±7.52	28.35±9.59	30.95±1.15
Duration of smoking (years)	13.93±12.00	9.27±7.10	–	–
Cigarettes/day	24.67±5.71	13.17±3.46	–	–

Table 2 Erythrocyte activities of SOD, GSH-Px and CAT in smokers and controls.

	Group I (heavy smokers)	Group II (light smokers)	Group III (passive smokers)	Group IV (controls)
SOD (U/gHb)	2009.2±272.2*	1922±239.6*	1902±284.4*	2389.8±436.6
CAT (k/gHb)	241.3±198.9*	344.6±170.8*	364.1±135.4*	555.5±194.7
GSH-Px (U/gHb)	22.95±11.72**	21.16±5.62**	32.57±9.34	27.01±8.13**

Values are mean±SD; *$p<0.01$ compared with group IV; **$p<0.01$ compared with group III

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Table 3 Relationship between enzyme activities and smoking status in smokers and controls (correlation coefficient).

Groups	Group I (heavy smokers)	Group II (light smokers)	Group III (passive smokers)	Group IV (controls)
SOD/GSH-Px	0.22	0.59[a]	0.44[c]	0.75[a]
SOD/CAT	0.01	−0.07	0.19	0.26
GSH-Px/CAT	0.27	−0.14	0.47[c]	−0.03
Number of cigarettes/CAT	−0.34[c]	−0.06	–	–
Number of cigarettes/SOD	0.00	−0.36[c]	–	–
Number of cigarettes/GSH-Px	−0.11	−0.29	–	–
Duration of smoking/CAT	−0.18	−0.02	–	–
Duration of smoking/SOD	−0.09	−0.40[c]	–	–
Duration of smoking/GSH-Px	−0.16	−0.49[b]	–	–

[a]$p<0.001$, [b]$p<0.01$, [c]$p<0.05$

groups II, III and IV ($r=0.59$, $p<0.001$; $r=0.44$, $p<0.05$ and $r=0.75$, $p<0.001$, respectively). Finally, there was a correlation between the activity of erythrocyte CAT and GSH-Px ($r=0.47$, $p<0.05$) in group III.

Discussion

Cigarette smoke contains many oxidants, free radicals and metastable products that are capable of reacting with, or inactivating, the essential cellular constituents. In addition, smoking causes an increase in oxidative metabolism of macrophages and neutrophils. The increased oxidative metabolism of phagocytes is accompanied by the increased generation of reactive oxygen species (2–5). Because these are potentially toxic, they usually are inactivated or scavenged by antioxidants before they can inflict damage to lipids, proteins or nucleic acids causing cellular dysfunction and even death. SOD, CAT and GSH-Px are generally believed to play vital roles in protecting the body against the toxic effect of oxidants (7).

We found significantly decreased erythrocyte SOD and CAT activities in active and passive smokers in comparison to non-smokers. There was a tendency towards decreasing SOD and CAT activity in the erythrocytes from group IV to group I. Erythrocyte GSH-Px activity was highest in passive smokers and lowest in light smokers. This decrease in enzyme activity most probably reflects the increased oxidative stress thought to occur in smokers. Breakdown of antioxidants may occur in cigarette smoking (13). While there was a positive correlation between the activity of SOD and GSH-Px in groups II and III and in the control group, there was no correlation in the heavy-smoker group which indicated abolished relationship between the activity of free radical metabolizing enzymes. GSH-Px and CAT complement each other in respect to intracellular location. CAT scavengers high concentrations of H_2O_2 more effectively, whereas GSH-Px is important as a scavenger at low concentrations (14). Generally, the change in CAT activity is closely related to the level of SOD because superoxides are converted to H_2O_2 by SOD or by spontaneous dismutation reaction (5). The enhanced susceptibility of erythrocytes in smokers to peroxidation may reflect lower activities of GSH-Px, CAT and SOD. Differences in GSH-Px activity between smokers and non-smokers have been reported previously and may be associated with the decreased selenium status (15). Smokers may be less sensitive to free radical damage than non-smokers because of changes in the levels of antioxidant enzymes. In passive smokers, increased GSH-Px activity exerts a protective effect from subsequent oxidant stress. This may explain why GSH-Px was higher in passive smokers than in active smokers.

Increased (7) and decreased erythrocyte antioxidant enzyme activity (8, 13) has been reported in smokers. Duthie *et al.* (6) reported that erythrocyte CAT and SOD activity was the same in smokers and non-smokers, while GSH-Px activity was lower in smokers. There were no effects of smoking on erythrocyte CAT and GSH-Px activity in another study (16). Hulea *et al.* (17) found higher erythrocyte GSH-Px and SOD activity in smokers in the 18–45 years age group and lower activity in the age of 46–80 years. In cigarette smokers, erythrocyte GSH level was significantly lower compared to healthy controls (18). It has been demonstrated that smoking depresses plasma antioxidant capacity (19, 20, 21), and smoking cessation is associated with decreased markers of oxidation in the plasma of active cigarette smokers (19). The authors of the study argue that this finding is consistent with the view that smokers are under a sustained oxidative load and that an inadequate antioxidant status combined with the increased free radical load caused by smoking may exacerbate the oxidation of LDL and increase its atherogenic properties (20).

Our results showing lower activity of SOD, CAT and GSH-Px in the erythrocytes are consistent with previous findings. Lower levels of antioxidant enzymes may cause the accumulation of free radicals generated by smoking. It has been demonstrated that a pro-oxidant-antioxidant imbalance exists in the blood of smokers. Generally, the redox status *in vivo* is determined by the balance between pro-oxidant and antioxidant capacity. Our data show clearly that exposure of humans to cigarette smoke results in the decrease of the activity of the major antioxidant enzymes.

In conclusion, an oxidant/antioxidant imbalance ex-

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ists in smokers and oxidative stress might be exacerbated. Passive smokers are affected by the environmental smoke as much as active smokers.

References

1. Kalra J, Chaudhary AK, Prasad K. Increased production of oxygen free radicals in cigarette smokers. Int J Exp Path 1991; 72:1–7.
2. Schumacher JM, Green CR, Best FW, Newell MP. Smoke composition: an extensive investigation of the water-soluble portion of cigarette smoke. J Agric Food Chem 1977; 25:310–20.
3. Sakuma H, Ohsumi T, Sugawara S. Particulate phase of cellulose cigarette smoke. Agric Biol Chem 1980; 44:555–61.
4. Hunninghake GW, Crystal RG. Cigarette smoking and lung destruction: accumulation of neutrophils in the lungs of cigarette smokers. Am Rev Respir Dis 1983; 128:833–8.
5. Sohn HO, Lim HB, Lee YG, Lee DW, Kim YT. Effect of subchronic administration of antioxidants against cigarette smoke exposure in rats. Arch Toxicol 1993; 67:667–73.
6. Duthie GG, Arthur JR, James WPT. Effects of smoking and vitamin E on blood antioxidant status. Am J Clin Nutr 1991; 53 Suppl 4:1061S–3S.
7. Brown KM, Morrice PC, Arthur JR, Duthie GC. Effects of vitamin E supplementation on erythrocyte antioxidant defense mechanism of smoking and non-smoking men. Clin Sci (Colch) 1996; 91:107–11.
8. Zhou JF, Yan XF, Guo FZ, Sun NY, Qian ZJ, Ding DY. Effects of cigarette smoking and smoking cessation on plasma constituents and enzyme activities related to oxidative stress. Biomed Environ Sci 2000; 13:44–55.
9. Van Kampen EJ, Zijstra WG. Determination of hemoglobin and its derivatives. Adv Clin Chem 1965; 8:141–87.
10. Sun Y, Oberley LW, Ying L. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988; 34:497–500.
11. Paglia D, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; 70:158–69.
12. Aebi H. Catalase. In: Bergmayer HU, editor. Methods of enzymatic analysis. New York: Weinheim Academic Press, 1974;647–83.
13. Godandabany U. Erythrocyte lipid peroxidation and antioxidants in cigarette smokers. Cell Biochem Funct 2000; 18:99–102.
14. Calabrese EJ, Canada AT. Catalase; its role in xenobiotic detoxification. Pharmacol Ther 1989; 44:297–307.
15. Ellis NI, Lloyd B, Lloyd RS, Clayton BE. Selenium and vitamin E in relation to risk factors for coronary heart disease. J Clin Pathol 1984; 37:200–6.
16. Björkman L, Svartengren M, Nordberg M. Individual differences in activity of glutathione peroxidase and catalase studied in monozygotic twins discordant for smoking. H Exper Toxicol 1992; 11:341–6.
17. Hulea SA, Olinescu R, Nita S, Crocnan D, Kummerow FA. Cigarette smoking causes biochemical changes in blood that are suggestive of oxidative stress: a case-control study. J Environ Pathol Toxicol Oncol 1995; 14:173–80.
18. Srour MA, Bilto YY, Juma M. Susceptibility of erythrocytes from non-insulin-dependent diabetes mellitus and hemodialysis patients, cigarette smokers and non smokers subject to *in-vitro* oxidative stress and loss of deformability. Clin Hemorheol Microcirc 2000; 22:173–80.
19. Petruzzelli S, Tavanti LM, Pulera N, Fornai E, Puntoni R, Celi A, *et al.* Effects of nicotine replacement therapy on markers of oxidative stress in cigarette smokers enrolled in a smoking cessation program. Nicotine Tob Res 2000; 2:345–50.
20. Duthie GG, Shortt CT, Robertson JD, Walker KA, Arthur JR. Plasma antioxidants, indexes of lipid peroxidation and coronary heart disease risk factors in a Scottish population. Nutr Res 1992; 12:61–7.
21. Pincemail J, Siquet J, Chapelle J, Cheramy-Bien J, Paulissen G, Chantillon A, *et al.* Determination of plasmatic concentrations in antioxidants, antibodies against oxidized LDL, and homocystein in a sampling of Liege population. Ann Biol Clin 2000; 58:177–85.

Received 6 December 2001, revised 3 April 2002, accepted 15 April 2002

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